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Mammalian neural stem cells.

Gage FH.

The Salk Institute, Laboratory of Genetics, 10010 North Torrey Pines Road, La Jolla, CA 92037, USA. gage@salk.edu.

Neural stem cells exist not only in the developing mammalian nervous system but also in the adult nervous system of all mammalian organisms, including humans. Neural stem cells can also be derived from more primitive embryonic stem cells. The location of the adult stem cells and the brain regions to which their progeny migrate in order to differentiate remain unresolved, although the number of viable locations is limited in the adult. The mechanisms that regulate endogenous stem cells are poorly understood. Potential uses of stem cells in repair include transplantation to repair missing cells and the activation of endogenous cells to provide "self-repair. " Before the full potential of neural stem cells can be realized, we need to learn what controls their proliferation, as well as the various pathways of differentiation available to their daughter cells.

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dramatic type of reprogramming is suggested by some recent experiments on the grafting of bone marrow cells between individuals. It has recently been shown that genetically marked bone marrow can contribute to the regeneration of skeletal muscle (*47*) and of liver (*48*) in the host animals. In one study, the graft was composed of purified hemopoietic stem cells (*49*). Although the frequency of labeled foci is small and the time for their development is long, this is still remarkable because it implies a much more extreme reprogramming of developmental commitment than that found in endogenous metaplasias. The experiments involve the injection of suspensions of cells, so single graft cells are likely to end up completely surrounded by cells of a foreign tissue. In embryological experiments, isolated single cells often show more developmental lability than extended masses of tissue (*50, 51*), so perhaps this should be expected in the adult animal as well.

The results of such experiments should not confuse us by suggesting that all types of stem cell are the same. The well-characterized hematopoietic stem cell is clearly quite distinct from the equally well studied early embryonic stem cell and probably equally distinct from the epithelial stem cells of the various differentiated tissue types. However, they do show that there is considerable potential scope for reprogramming epithelial stem cells by changes to their environment.

The existence of endogenous processes of tissue repair in many or most epithelia suggests that there is a whole unexplored area of potentially novel therapies based on the stimulation of these regenerative mechanisms. Progress will require better characterization of epithelial stem cells in terms of molecular markers. It will also require the establishment of more in vitro culture systems, like those used for epidermis (*3, 52*), in which the control of stem cell behavior can be investigated in detail. Perhaps the most important advance will be the identification of the mysterious environmental factors that control stem cell behavior, both with regard to self-renewal potential and to the ability to form particular types of differentiated cells.

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REVIEW

Mammalian Neural Stem Cells

Fred H. Gage

Neural stem cells exist not only in the developing mammalian nervous system but also in the adult nervous system of all mammalian organisms, including humans. Neural stem cells can also be derived from more primitive embryonic stem cells. The location of the adult stem cells and the brain regions to which their progeny migrate in order to differentiate remain unresolved, although the number of viable locations is limited in the adult. The mechanisms that regulate endogenous stem cells are poorly understood. Potential uses of stem cells in repair include transplantation to repair missing cells and the activation of endogenous cells to provide "self-repair." Before the full potential of neural stem cells can be realized, we need to learn what controls their proliferation, as well as the various pathways of differentiation available to their daughter cells.

The term "neural stem cell" is used loosely to describe cells that (i) can generate neural tissue or are derived from the nervous system, (ii) have some capacity for self-renewal, and (iii) can give rise to cells other than themselves through asymmetric cell division. Whether stem cells from neural and other tissues are more defined by their tissue of origin or by their multipotentiality is at present unclear. However, neural stem cells can also be derived from more primitive cells that have the capacity to generate neural stem cells and stem cells of other tissues (Fig. 1). Stem cells have varying repertoires. A totipotent stem cell can be implanted in the uterus of a living animal and give rise to a full organism, including the entire central and peripheral nervous systems. A pluripotent

The Salk Institute, Laboratory of Genetics, 10010 North Torrey Pines Road, La Jolla, CA 92037, USA. E-mail: gage@salk.edu

stem cell is restricted in that it can give rise to every cell of the organism, including cells of the nervous system, except the trophoblasts of the placenta. Stem cells out of context are not able to give rise to the form and structure of the organism. The pluripotent cell is the same as an embryonic stem cell (ES cell) and is currently used to create transgenic animals; it is also the one being proposed for use in a wide variety of commercial and clinical applications (*1*). Most stem cells fall into the category of multipotent stem cells, a term that really does not provide much useful information, because the developmental potential of these cells has usually not been fully tested. Most often, stem cells are defined by the organ from which they are derived or by where they are observed in vivo. The assumption in recent decades has been that an authentic stem cell from one of these organs can give rise to all cells of that organ and to only cells of that organ. It is the challenge to this latter assumption that has recently generated such excitement.

In mammals, the diversity of structures, functions, and cell types in the nervous system makes the study of stem cells more difficult than in organisms like *Drosophila* (*2*). For the mammalian nervous system, it is unknown whether or not stem cells from different regions of the brain carry different constraints. In fact, it is not clear whether stem cells obtained from a given region of the embryonic brain are different from those derived from the structure in the adult brain that the embryonic region gave rise to. The nervous system is unlike the hematopoietic system, wherein the functional requirements of self-renewal and multipotency of the stem cell during development are assumed to be similar to those of the adult, because of the need for constant replenishment of the blood system.

The observation of stem cells in the adult nervous system has not been adequately integrated into our ideas of the function of the adult brain, which had long been thought to be entirely postmitotic. The importance of long-term, regular cellular self-renewal in the central nervous system is uncertain. In the absence of a defined function for these adult stem cells, it has been suggested that they are vestiges of evolution from more primitive organisms, like planaria or fish (*3*), in which organ and tissue self-renewal provides survival advantages in an inhospitable environment. An alternative view is that the adult mammalian nervous system retains a limited capacity for self-renewal that is important for its normal functions, like learning and memory. It is possible that the local generation of new neurons in structures could participate in the formation or integration of new memories. The ability of adult neurogenesis to be regulated by changes in the environment further supports a role in normal behavior. The implications would be that the brain controls behavior and behavior can change the structure of the brain.

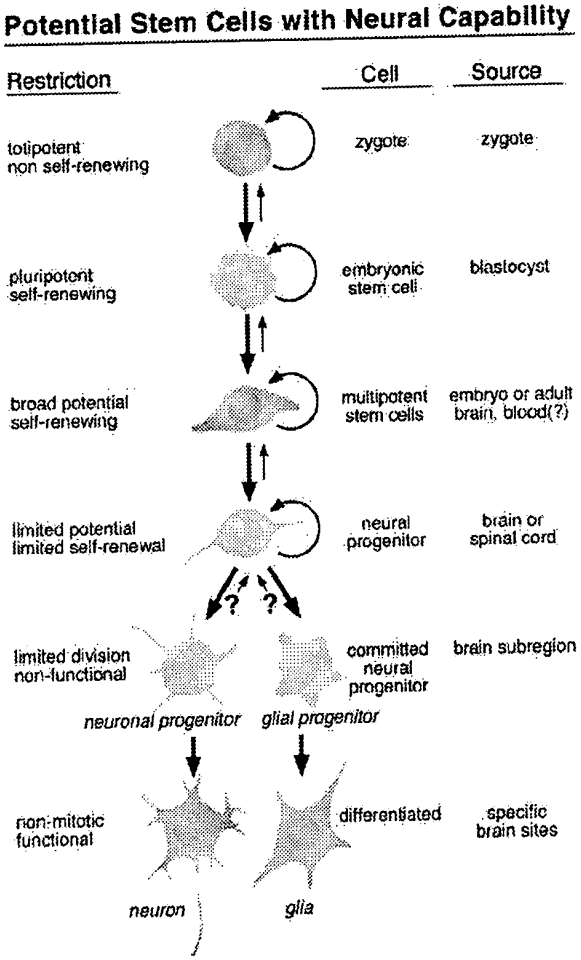

Fig. 1. An illustration proposing the classes of mammalian stem cells that can give rise to neurons, presented as a hierarchy beginning with the most primitive and multipotent stem cell and progressing to the most restricted. The restrictions of fate at each step and examples of sites in the body where they can be obtained are also presented. As our understanding of the true potential and nature of stem cells is still unfolding, modifications will clearly be added. For example, the small arrows pointing up suggest the potential, although not well documented, dedifferentiation of the more restricted cell below.

How Are Neural Stem Cells Investigated?

Stem cells in vitro. The standard method of isolating neural stem cells in vitro is to dissect out a region of the fetal or adult brain that has been demonstrated to contain dividing cells in vivo, for example, the subventricular zone (SVZ) or the hippocampus in the adult or a larger variety of structures in the developing brain. Usually, the tissue is disaggregated and then the dissociated cells are exposed to a high concentration of mitogens such as fibroblast growth factor–2 (FGF-2) (*4*) or epidermal growth factor (EGF) (*5*) in either a defined or supplemented medium on a matrix as a substrate for binding. After some proliferation, the cells are either induced to differentiate by withdrawing the mitogens or by exposing the cells to another factor that induces some of the cells to develop into different lineages. Cellular fates are analyzed by staining with antibodies directed against antigens specific for astrocytes, oligodendrocytes, and neurons. In some cases, cells are plated at low density and monitored to determine if a single cell can give rise to the three phenotypes (*6*). Stem cell properties can be further demonstrated when cells are lineage tagged with a retrovirus in vitro, after which the clones of cells derived from the original tagged cell are proven to have been derived from a single cell by Southern analysis (*7*).

Despite the similarities in methods used, there are big differences reported by various laboratories in the procedures used to manipulate stem cells, which could account for the discrepancies in their results; for example, some investigators use EGF as a primary mitogen to expand the most primitive cells, whereas others use FGF-2 with or without EGF. The species under study, mouse or rat, varies, and studies rarely control for the strain of rodents. Technical

issues—such as the region dissected, the dissection method, age of donor, cell density, substrate used for coating the plates, and whether the cells are expanded as floating aggregates (called "neurospheres") (*5*, *6*) or as monolayers attached to the culture dish surface—potentially play important roles in what is defined as a stem cell. In some cases, cells are infected or transfected with oncogenes such as simian virus 40 large T antigen (*8*) or *v-myc* (*9*, *10*) to facilitate subsequent proliferation, but these "oncogenetically immortalized cells" are probably genetically altered and are subject to additional mutations that will render them either tumorigenic or unuseful for studies of the normal genes involved in lineage analysis and fate determination. Although most studies have used rodent cells, recent studies have reported that human fetal tissue is also a source of neural stem cells (*10*, *11*).

Defining a population of cells in vitro as stem cells presents inherent problems, including, most importantly, the demonstration that the cells retain the capacity to fully develop into all of the mature fates of the cells for which the putative stem cell is supposed to be a precursor. Thus, although until recently it has been adequate to use a single antibody marker to demonstrate that a cell is a neuron (TUJ1), an astrocyte [glial fibrillary acid protein (GFAP)], or an oligodendrocyte (GalC), there are hundreds of different types of neurons, and it will be important to distinguish which cells of the lineage they can become. Now that some stem cells can be induced to differentiate toward specific cell lineages, identifying the signal cascades that mediate these fate choices has become a major field of investigation (*12*).

Transplantation of characterized stem cells in vivo. To determine more completely the fate potential of stem cells that are characterized in vitro, investigators have grafted cells expanded with mitogenic growth factors and/or genetically marked cells back to the brain. In some cases, the fetus-derived stem cells are grafted to the developing brain to determine the range of cell types that the grafted cells can differentiate into.

The range of the surviving cell types that these grafted, expanded, nervous system stem cell populations can differentiate into is greater than that anticipated. Not only can cells migrate broadly throughout the developing brain and peripheral nervous system, but also populations containing stem cells derived from the human fetus can be implanted in the adult rat brain where they differentiate into neurons and glia (*13*). The fate of the grafted cells appears to be dictated by the local environment rather than the intrinsic properties of the cells themselves. Thus, when grafted to the developing brain, fetus-derived stem cells and immortalized progenitor cells migrate along with the host cells and differentiate into cell types specific for the target region (*14*). The ability of the implanted stem cells to react appropriately to local signals in the normal developing brain results in chimerism, with the grafted cells being indistinguishable from the host cells in the best instances. In damaged developing brain tissue, immortalized cells have been shown to migrate to areas of damage, where they replace depleted cells (*15*).

This remarkable plasticity is not, however, limited to the developing brain. Stem cells obtained from the adult hippocampus can be expanded in vitro and implanted back into the hippocampus, where they generate new neurons and glia, similar to the cells they generate normally in the adult dentate gyrus (*16*, *17*). Furthermore, these same cells can generate olfactory bulb neurons when implanted in the rostral migratory stream (RMS), expressing neurotransmitter phenotypes, such as tyrosine hydroxylase, which the cells do not make in the hippocampus but which are normally generated in the olfactory bulb (*16*, *17*) (Fig. 2, A through D). When implanted into regions that do not normally generate neurons in the adult (for example, into the intact cerebellum or the striatum), the stem cells do not make neurons, but they do make glial cells, which are generated during injury (*16*, *17*). Most striking is the report that genetically marked mouse cells derived from the embryonic or adult brain and expanded in vitro as spheres were transplanted to an irradiated host mouse and gave rise to blood cell types, including myeloid and lymphoid cells and other more primitive hematopoietic cells (*18*). These results suggest that the potentiality of neuronal stem cells may not only extend beyond the region of the brain from which they are derived, but also may not be restricted to the brain at all.

Although these and many other studies confirm the range of cells that the grafted stem cells can differentiate into, there are clear caveats that need to be inserted into the conclusions drawn from these studies. One is that, at the time of grafting, when the cells are expanded to a population size adequate to track them in vitro, there is a complex mixture of cells at various stages of maturation, and only a small fraction of the grafted cells, if any, retains stem cell properties. Thus, the cells that do differentiate into more mature cells in vivo may already have differentiated in vitro to a certain extent, and the most immature cells may either not survive or remain quiescent. This problem can be overcome to some extent by "serial grafting" (*19*), demonstrating that some of the surviving grafted cells are self-renewing. In serial grafting, the labeled cells are grafted and then harvested from the host brain again, expanded, cloned, and then implanted into another host brain. Another concern about the interpretation of studies that purport to show multipotentiality of cells after in vitro proliferation is possible dedifferentiation or other genetic modification of the cells due to extended exposure to mitogens. Indeed, brief exposures to high concentrations of FGF-2 in vitro permitted neurogenesis in vitro in stem

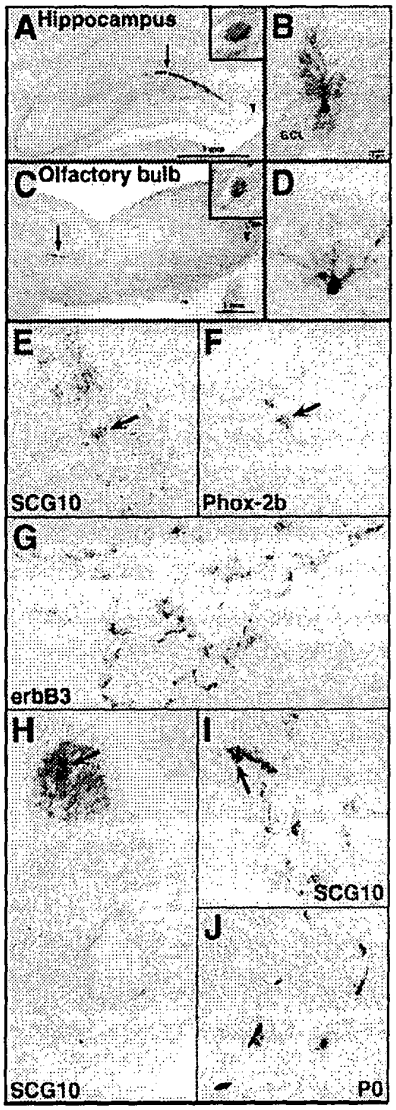

Fig. 2. Examples of propagated and genetically marked adult hippocampal cells grafted to (**A** and **B**) the adult hippocampus and (**C** and **D**) the RMS, where they differentiate into neurons appropriate to the target area [modified by permission from *Nature* (*17*)]. (**E** through **J**) Neural crest stem cells that were isolated and enriched in vitro and then grafted to a host animal, demonstrating the multipotent capacity of the freshly isolated stem cells [reprinted by permission from (*21*), copyright 1999 Cell Press].

cells isolated from nonneurogenic regions of the adult brain (*20*).

An additional concern is that the identity of the stem cell is inferred from the procedures. At present, no individual neural stem cell from the central nervous system has been identified and isolated adequately to separate it unambiguously from other, more committed cells in vitro or in vivo. Markers are needed that can identify stem cells in their most primitive state. The most complete characterization of nervous system stem cells was accomplished by Morrison *et al.* (*21*), who used fluorescence-activated cell sorting to achieve an enrichment of 80% of the cells that could differentiate into all neural crest lineages. The identity of the sorted cells was proven by grafting them back to a host animal, where the isolated cells differentiated into the appropriate phenotypes (Fig. 2, E through J).

The major obstacle to identifying and discovering markers that define a stem cell is that the most primitive cells are probably in a quiescent state and do not express many unique antigens. Thus, as with other fields like hematopoiesis, a combination of positive and negative markers will be required to better define the central nervous system stem cell. However, we must acknowledge that even this approach of analyzing multiple markers has not yet identified the consensus bone marrow–derived stem cell (*22*).

Neural cells can be derived from more primitive cells, including ES cells (Fig. 1). Specifically, Brüstle *et al.* (*23*) showed that mouse ES cells could be induced to differentiate into a mixed population of cells enriched for oligodendrocyte precursors. These enriched cultures were then implanted in the spinal cords of myelin-deficient rats depleted of endogenous oliogodendroglia, whereupon the ES cell–derived oliogodendroglia precursors migrated widely and ensheathed demyelinated axons, ultimately developing to appear similar to host mature oligodendrocytes. Whether grafted cells are functional and whether functional neurons can also be generated from ES cells in vitro remain to be determined. In another study, mouse ES cells were induced to partially differentiate in vitro before transplantation in a rat model of spinal contusion (*24*). The authors reported a modest but significant improvement in the level of behavior that the grafted animals attained in relation to controls, and the cells survived for up to 5 weeks after grafting. However, the role that the grafted cells played in the recovery, whether supplying trophic factor support or contributing to cellular reconstitution, was not explored (*24*).

Mesenchymal stem cells of the stroma have been examined for their ability to generate cells of the neural lineage, but with less success. Cells appear to survive when they are implanted in the brain and then migrate broadly. Some of the cells may differentiate into astroglia, but additional treatment in vitro to enrich, instruct, or select for neural lineage cells may be needed to achieve neurons from mesenchymal stem cells (*25*).

These studies suggesting reciprocity between cells of different lineages raise the specter that cells of the brain may not be derived from the brain. Where do they come from?

In vivo stem cells. Stem cells are often detected in vivo, through the use of retroviruses (*26*) or with thymidine or bromodeoxyuridine (BrdU) labeling (*27*). Retroviruses infect only dividing cells, can be passed on to all progeny of the infected cells, and reflect a particular cell's lineage when the probability of infecting two closely adjacent cells is low. However, this procedure is generally inefficient and nonquantitative. In addition, retroviral expression is most often down-regulated with terminal differentiation, so the full range of cell phenotypes may be underrepresented. Labeled nucleotide substitution methods with BrdU and thymidine can reveal the total numbers of cells dividing at any time, but if cells continue to divide, the label will be diluted. In addition, caution is required to be certain that the labeled nucleus exists in the cell of interest nearby (*28*) and that labeling is not attributable to DNA repair. Thus, although it is possible to determine rather precisely when cells are born and whether or not multipotent cells exist at a particular time, determining how multipotent the cells are or whether the cells are self-renewing is not yet possible in vitro. Despite these concerns, important insights have been gained into the endogenous proliferation of cells and their fates in the developing brain and spinal cord (*29*) and, more provocatively, in the adult brain and spinal cord. Following Altman's pioneering work with thymidine labeling in the adult rat brain (*27*), one of the earliest and best characterized examples of adult neurogenesis is that of the songbird forebrain (*30*). Widespread cell proliferation and migration have been documented, along with differentiation into new neurons in the dorsomedial caudal neostriatum, an area associated with song learning. The neurons that are formed differentiate into physiologically functional neurons within the local circuit, in some cases establishing long-distance projections (*31*).

Factors regulating endogenous stem cells. After some years of debate, it is now accepted that, in all adult mammalian brains, there are two sites of high-density cell division: the SVZ and the subgranular zone (SGZ) of the dentate gyrus of the hippocampal formation (*32*). The exact phenotype of the most primitive cell in these areas is not yet known, but a recent set of papers clearly documented the complexity of this seemingly straightforward question. Johansson *et al.* (*33*) provided evidence that a subpopulation of ependymal cells in the lining of the third ventricle was the stem cells. Subsequently, Doetsch *et al.* (*34*) presented more convincing evidence that a subset of cells in the SVZ was stem cells and that these cells expressed GFAP, a marker of astrocytes, suggesting that stem cells in this region of the brain are related to astrocytes (Fig. 3, D through F). Meanwhile, a third group (*35*) dissected the ependyma from the subependyma and found that, although both cells could divide in culture, only the subependyma-derived cells could self-renew and give rise to neurons and glia.

Definitive identification will require phenotypic markers that discriminate between different cell types or different states of a common cell. Once a stem cell divides asymmetrically, the more mature progenitor is born and migrates to regions of differentiation. As the progenitor migrates, it matures further until it reaches a site where it stops and either becomes quiescent or fully differentiates into a functioning cell (*12, 36*).

Defining the diffusible factors and substrate-bound molecules that guide this process constitutes one of the most active and exciting areas of developmental biology at present, and many of the molecules that have been found to be important in the developing brain persist in the adult brain in areas where neurogenesis continues. However, some differences may exist between mechanisms of migration in the adult and developing brain. For example, the daughter cells for the SVZ migrate long distances in the RMS, to the olfactory bulb where they integrate as neurons (*37*). There are, however, no radial glia on which progenitors can migrate, so they use a novel cellular process called "chain" migration, which involves homotypic interactions between the migrating cells and tubelike structures formed by specialized astrocytes (*38*) (Fig. 3, D through F). As in development, however, a highly polysialated glycoprotein neural cell adhesion molecule (PSA NCAM) is present in this migratory stream on the surface of the migrating cells, and deletion of the gene for NCAM or cleavage of the polysialic acid moiety results in defects in migration and reduction in the size of the olfactory bulb (*39*). This same molecule is also present in the dentate gyrus on the surface of newborn adult progenitor cells as they migrate from the SGZ

into the granular layer proper. As the granule cells mature and stop migrating, they no longer express PSA NCAM. The dentate gyrus is also reduced in size in the NCAM knockout mice (*40*). Thus, depending on cell age, the distance required for migration, and the type of cell, both common and novel mechanisms for migration can be used. Less is known about the mechanism of differentiation or the function of the newly born neurons in the adult brain, but some of the factors that regulate proliferation, migration, survival, and differentiation have been investigated in dentate granule cells (Fig. 3, A through C). In the adult macaque monkey, neurogenesis continues in the prefrontal, inferior temporal, and posterior parietal cortex, but not in primary sensory areas like the striatal cortex (*41*). The extent to which this observation represents a significant functional population of cells and whether this is unique to primates will be important in interpreting the importance of neurogenesis in the adult brain.

Although the exact kinetic studies are lacking in the adult brain, a conservative estimate for rat and mouse suggests that 1 neuron is produced each day for every 2000 existing neurons (*42*). The rate of neurogenesis declines with age, but neurogenesis persists in the dentate gyrus in elderly rodents and humans (*27*). Although evidence of increased cell death in the dentate has been suggested and the exact relation between the birth of new neurons and the death of older ones is not known, there is an assumption of some balance between the two. More cells are born in the dentate of the adult than survive, but the rate of survival can be greatly increased by housing either young adult or aged animals in "enriched environments" (*43*). Genetics also strongly influences neurogenesis in this region (*42*), but the effects of an enriched environment can compensate for differences in neurogenic rates in the dentate gyrus of at least two strains of adult mice (*44*). The exact elements of the enriched environment that are critical to the survival effect are not known, but two reports suggest that learning of a specific type of task can influence rates of survival. More dramatically, voluntary exercise can nearly double the number of proliferative cells as well as the number that survive as neurons (*45*). Furthermore, voluntary exercise not only affects neurogenesis in the dentate gyrus, but it can also selectively increase the amplitude of long-term potentiation in the dentate gyrus, but not in the CA1 of the same animals, showing a functional correlate for these new neurons in the brain (*46*).

Some specific regulators of neurogenesis have been identified, although the mechanisms through which they act are not known. The inhibitory role of glucocorticoids on neurogenesis is best characterized by the adrenalectomy-induced increase in proliferation and the antagonism of this effect with systemic application of glucocorticoids (*47*). The effects of the glutamatergic system are less clear but equally great. One group reported that glutamatergic deafferentiation causes an increase in all aspects of neurogenesis and the glutamatergic receptor antagonist MK-801 also increases proliferation (*48*). However, in apparent contrast, experimentally induced temporal lobe seizures induced by excitatory amino acids cause a dramatic increase in proliferation and neurogenesis in the dentate gyrus (*49*). This latter effect appears aberrant, because the neurons that are formed do not send processes to the CA3 region of the hippocampus, as is observed for ongoing neurogenesis (*50*), but rather the epilepsy-induced neurogenesis sends axon collaterals back onto the dentate gyrus, forming recurrent collaterals. Thus, although "normal" neurogenesis can be enhanced and correlated with enhanced function, neurogenesis can be recruited abnormally, resulting in a correlation with aberrant function.

Growth factors that have been shown to have an effect on stem cell proliferation in vitro have also been shown to influence the behavior of endogenous cells. When recombinant EGF and FGF-2 were infused in the lateral ventricle system of adult rats and mice (*51*), EGF strongly increased proliferation of cells in the SVZ, but not in the SGZ, and influenced the fate of the cells in the SGZ, resulting in more glial cells and fewer neurons. The effects of intraventricular FGF-2 in the adult were less dramatic, but when FGF-2 was injected systemically in the neonate, substantial increases in neurons were observed in the brain, through an as yet unknown mechanism (*52*). Brain-derived neurotrophic factor injected in the ventricle of mice increased the number of cells and probably the number of neurons in the olfactory bulb, and other fac-

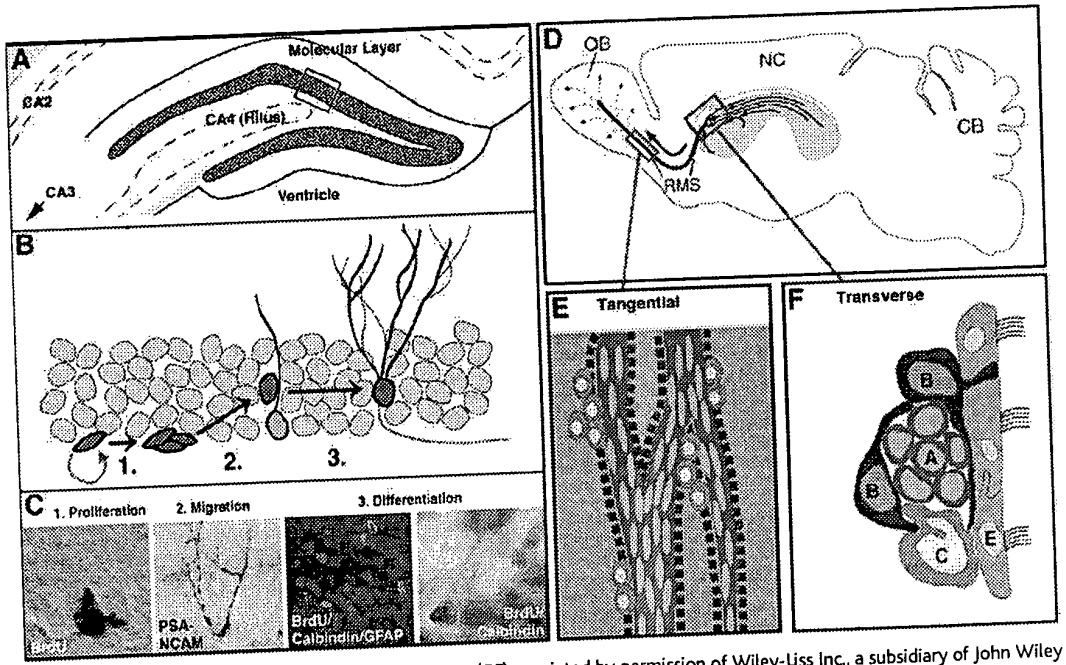

Fig. 3. Examples of the origin and migratory pattern of the stem cells observed in situ in (**A** through **C**) the adult dentate gyrus and (**D**) the subependymal RMS. The box in (A) indicates the granule cell layer illustrated in (B). The numbers in (B) refer to the three steps shown in (C). OB, olfactory bulb; NC, neocortex; CB, cerebellum. (**E**) A schematic of the chain migration of the cells in the RMS, which originate in the subependymal zone. (**F**) The red cells (A label) are the neural progenitor cells, the purple (B label) cells are astrocytes, and the green (C label) cells are precursor cells that are found scattered along the RMS (E label). (A) through (D) are a composite by H. G. Kuhn, and (D) through (F) are from (*55*), reprinted by permission of Wiley-Liss Inc., a subsidiary of John Wiley & Sons, Inc.

tors are now being examined for their effects (*53*).

The large number of genetic, environmental, and molecular factors that can regulate various aspects of the proliferation, migration, and differentiation of adult stem cells in vivo suggests that the function of these newly born cells may be quite broad and relevant to a variety of fundamental and dynamic processes in the brain.

Prospective

It is difficult to speculate what the future will reveal about neural stem cells. It may turn out that neural stem cells are derived systemically or, more likely, that systemic stem cells and their progeny have a dramatic effect on the behavior of neural stem cells.

Given the excitement in the research community about neural stem cells, we can expect that interesting new observations will be rapidly replicated, and the knowledge about stem cells will be applied quickly, and hopefully safely and effectively. From what we know already, isolated fetus- or adult-derived neural stem cells from mouse, rat, and human brain tissue survive well in the developing and adult, intact and damaged, brain and can migrate over sizable distances, in some cases to copopulate or repopulate brain regions undergoing changes. Whether the stem cells take on the exact function of the cells they replace or displace remains to be determined, and the answer will be the foundation on which therapeutic strategies will be built. The stem cells may need to be genetically engineered to induce their differentiation toward specific lineages, or more likely, the cells that integrate into a particular circuit will need training by neighboring cells to function appropriately. This latter suggestion implies that cellular transplantation in the absence of training of the newly transplanted cells might be less effective, as suggested by recent fetal tissue grafting experiments (*54*). An alternative therapeutic application of stem cells is based on the fact that neurogenesis continues in the adult and that this neurogenesis can be regulated by many factors. The extent to which knowledge of regulators of endogenous neurogenesis can be defined will determine whether a strategy of self-repair or endogenous repair can be achieved, or enhanced if it is being used now, as a complementary therapy in the future.

Fundamental questions remain concerning neural stem cell biology. Where are adult neural stem cells located—in the brain, in blood, or in both, or in other tissues as well? Are there definitive ways to identify a neural stem cell and distinguish it from other sorts of stem cells? Do mitogens, oncogenes, or isolation in vitro change the potential of the neural stem cell? Are there limits to where or when neurogenesis can occur in the brain or spinal cord? What are the mechanisms that determine whether a stem cell will divide symmetrically or asymmetrically, differentiate into a neuron or a glial cell, become quiescent, or die? What are the functions of the new neurons born in the adult brain? The answers to these questions will greatly accelerate therapeutic applications.

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